Academy of Romanian Scientists
Annals Series on Biological Sciences

Volume 14, No. 1, 2025, pp. 226-235
Online ISSN 2285 – 4177
ORIGINAL PAPERS

GUT MICROBIOME IMBALANCES IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

Daniela BOBOC[1], Natalia ROȘOIU[2]

[1] School Center for Inclusive Education "Maria Montessori" - Constanta, email: barbulet.daniela@yahoo.com
[2] Ovidius University of Constanta, Faculty of Medicine, Academy of Romanian Scientists, email: natalia_rosoiu@yahoo.com
*Corresponding author e-mail: barbulet.daniela@yahoo.com

Abstract. *Autism is a serious disorder of normal developmental processes that appears in the first two years of life. It affects language, cognition, social development and adaptive skills, causing increasing delays compared to children of the same age. The number of children diagnosed with Autism Spectrum Disorders is increasing, which is very worrying. The gut microbiome plays an important role in everyday life and ensures the proper functioning of the human body. In recent years, promising research has been conducted to better understand autism, including its possible causes or the development of new treatments and interventions. Many scientists are interested in how the gut microbiome might be linked to autism spectrum disorder. We conducted a study to identify whether there is a causal link between intestinal transit disorders, namely chronic constipation, the presence of behavioral disorders and the existence of an imbalance in the intestinal microbiome. In order to participate in this research, parents of children with autism spectrum disorders signed a consent agreement. Intestinal microbiome analyses were performed for 16 children and young people diagnosed with autism spectrum disorders, aged between 3 and 17 years. The results obtained were analyzed using the SPSS 20 statistical program. Children and young people with ASD participating in the research have imbalances in the intestinal microbiome. This can also be influenced by inadequate nutrition and further research should be conducted to emphasize the connection between nutrition and the microbiome. Due to inadequate nutrition, specific nutrients important for effective learning, social and intellectual development may be missing. Children with ASD tend to have a limited food repertoire and a greater reluctance to eat certain foods compared to neurotypical children. Modulating the gut microbiota in individuals with ASD and gastrointestinal disorders should be a promising target for future medical research. Diet plays an important role in determining the composition and function of the gut microbial community; therefore, a selective diet may influence the gut microbial community. Probiotics have recently been used in several clinical trials as an adjunct to conventional therapy in patients with autism spectrum disorders and we believe that they have a beneficial role for the gut microbiome.*

Keywords: Autism Spectrum Disorders, gut microbiome, intestinal transit disorders, deviant behaviors

DOI 10.56082/annalsarscibio.2025.1.226

Introduction

The problem of autism is extremely serious and topical. Although the number of children born with autism has increased, researchers have not yet identified the precise causes of autism. Autism is part of a class of pervasive developmental disorders, along with Rett disorder, childhood disintegrative disorder, Asperger's disorder, and pervasive developmental disorder not otherwise specified. To better understand the structure of the microbiota of children of all ages with autism spectrum disorders, it is important to evaluate the microbial population. Gut microbes and their interactions may be associated not only with metabolic problems, but also with behavioral symptoms of autism spectrum disorders. In particular, we focus on how imbalances in the microbiota can affect the health of people with autism spectrum disorders.

1. Conceptual Boundaries in Autism

Autism is a serious disorder of normal developmental processes that appears in the first two years of life. It affects language, cognition, social development, and adaptive skills, causing increasing delays compared to children of the same age. The cause is unknown, but research suggests physiological causes such as neurological abnormalities in certain areas of the brain (1). Studies examining the phenomenon of autism have concluded that several factors are responsible for the increase. The prevalence of autism spectrum disorders (as determined by current diagnostic methods) appears to be about five to six per 1,000 young children. In the first years of life, there are usually no clear defining features, but parents need to be vigilant. In the second and third years of life, a rapid examination by specialists is necessary for the following areas of concern: communication, language development, especially comprehension, disturbance, unusual use of language, difficulty responding to name, non-verbal communication - insufficient (without pointing with the hand and difficulty following a point), no social smile to share good mood and respond to the smile of others (2). Autism is an uncritical way of thinking, based on subjectivity and detached from reality, dominated by fantasy and dreams. The child with autism refuses contact with external people and objects and escapes into his inner world, where he satisfies his imaginative desires, emotionally compensating for fantasies, which in pathological cases lead to hallucinatory illusions (3). Autism is not a disease but a pervasive developmental disorder, a concept that better expresses what happens to these people and what are the stages in the application of therapy. This pervasive developmental disorder requires lifelong care facilities specialized in autism (4). While the etio-pathogenesis of ASD is unknown, the clinical manifestations are diverse and many possible genetic and environmental factors

have been implicated. As such, it has been a great challenge to identify key neurobiological mechanisms and develop effective treatments (5). "This disorder appears before the age of 2-3 years, but can be detected even in the first year of life. At birth, the child is apparently normal, without physical or neurological abnormalities. If the onset of autism is early – in the first year of life, then there is a lack of waiting movements while being held in the arms, lack of response to the mother's smile, apathy and lack of interest in what surrounds them" (6). Autistic children seem to not understand verbal and non-verbal communication and are characterized by varying degrees of isolation from the world around them. They have their own way of learning. They create an exaggerated interest in certain activities and objects, which hinders the development of play. They have very little interest in other children and usually do not learn by observing and imitating others. Although the learning process is very affected, behavioral analysis specialists are developing effective learning methods for children with autism based on the principles of learning theory (7). The strong correlation of gastrointestinal symptoms with autism severity indicates that those children with more severe autism are likely to have more severe gastrointestinal symptoms, and vice versa. It is possible that autism symptoms are exacerbated by the gastrointestinal problems they have (8).

2. The Gut Microbiome and its Importance

Despite its intrinsic complexity, the microbiome is partly inherited, in a process that likely involves the dynamics of power law construction in the "small world" in newborns (9). Research on the human microbiome has moved from a new field to a booming area of medical research, with over $1.7 billion spent in the past decade alone (10). Each person has a unique microbiome, similar to a fingerprint. The gut microbiome plays an important role in everyday life and ensures the proper functioning of the human body. In recent years, promising research has been conducted to better understand autism, including its possible causes or the development of new treatments and interventions. Many people are interested in how the gut microbiome might be linked to autism spectrum disorder. The term microbiome is often used to define the composite genome of microorganisms, while the term microbiota refers more specifically to the microorganisms themselves. In fact, the two terms are often used interchangeably (11). There is growing evidence that the microbiota-gut-brain axis can influence gastrointestinal health and modify behaviors associated with ASD. The gut microbiota and the metabolic products of microbial populations may be linked not only to gastrointestinal problems but also to behavioral symptoms in autism. In particular, we focus on how gut dysbiosis may affect intestinal epithelial permeability, immune function, and microbial metabolites in individuals with

autism. To better understand the structure of the gut microbiota in children with autism spectrum disorders of different ages, as well as the relationship between the gut microbiota and fecal metabolites, we need to assess the gut microbial population (12).

Microbiome analysis is extremely important for individuals with autism. Recently, the medical community has become increasingly aware of the importance of the populations of microorganisms that colonize the human gut, collectively known as the microbiota. While microbiota can refer to all microorganisms present in an environment, including bacteria, viruses, and fungi, the microbiome is defined by the collection of genomes (genetic material) from all microorganisms present in that environment. Recent studies have linked imbalances in the composition of the microbiota not only to inflammatory bowel disease but also to chronic diseases such as depression, autism, obesity, multiple sclerosis, cancer, rheumatoid arthritis, Parkinson's disease, and more (13). An imbalance of the maternal flora during pregnancy with insufficient colonization of the fetal flora can lead to the development of neurological diseases such as autism or schizophrenia. There is a bi-directional communication between the gut microbiome and the central nervous system (CNS) that is achieved through endocrine, nervous and immune signaling mechanisms called the gut-brain axis, the composition and functions of the gut microbiome being affected by brain activity through the autonomic nervous system (ANS) that can modify intestinal transit through secretion, regional intestinal motility and intestinal permeability (14).

Possible treatments for autism spectrum disorders include medications that were developed to treat other conditions that interfere with some of the symptoms of autism. Microbiome analysis may shed light on autism. Gastrointestinal disorders can affect the health of a child diagnosed with ASD, worsening certain symptoms, which can lead to problems with school integration. Diet plays an important role in determining the composition and function of the gut microbial community; therefore, a selective diet can influence the gut microbial community. Probiotics have recently been used in several clinical trials as an adjunct to conventional therapy in patients with autism spectrum disorders. The evidence for the use of metabolic disease therapies, such as low-carbohydrate diets, in the treatment of individuals with ASD should be examined to evaluate diet as a therapeutic approach to improve symptoms of autism spectrum disorder (15).

3. Research methodology

3.1. Research purpose and objectives

Research purposes

We aimed to identify whether there is a causal link between intestinal transit disorders, namely chronic constipation, the presence of behavioral disorders and the presence of an imbalance in the intestinal microbiome bioindicators.

Objectives

O1. Performing fecal microbiome analysis in study participants. O2. Identifying how intestinal imbalance influences the severity of symptoms. O3. Determining the values of bacteria in the intestinal microbiome in participants in this research.

3.2. Research hypotheses

Hypotheses

HS1. It is assumed that there are imbalances in the intestinal microbiome in participants with ASD. Hs2. It is assumed that the presence of transit disorders such as constipation may be a cause of changes in the intestinal microbiome. Hs3. It is assumed that disruption of the intestinal bacteria profile may influence behavioral manifestations.

3.3. Description of the subject group

A total of 16 children, diagnosed with ASD who presented behavioral disorders and intestinal transit disorders such as constipation, participated in this study. The children were diagnosed with infantile autism and severe developmental delay before the age of 3 and began psychological and speech therapy immediately after diagnosis at the Psychological Centers in Constanta. The ages of the participants in this study are between 3 and 17 years old and all live in Constanta. Fecal samples were collected in the Bioclinica laboratories in Constanta in order to analyze the intestinal microbiome for the study participants, which were subsequently sent for interpretation in Germany.

3.4. Ethical approval

To participate in this research, the parents of children with ASD signed a consent agreement.

3.5. Inclusion and exclusion criteria

Inclusion criteria:

- diagnosis of ASD;
- informed consent signed by parents;

- constipation
- presence of behavioral disorders.

Exclusion criteria:

- absence of autism diagnosis;
- absence of transit disorders;
- absence of consent agreement.

3.6. Presentation and interpretation of results

Table 1. Individual values for LPS positive bacteria

Nr.cr/ I.N.	Citrobacterspp. <0,001	Enterobacterspp. < 0,007	Escherichia spp. <0,003	Klebsiella spp. < 0,002	Pseudomonas spp. < 0,002	Serratiaspp. < 0,001	Sutterellaspp. < 2,9
1.R.D.C.	0,000	0,296	0,674	0,443	0,000	0,002	0,019
2.O.Mr.	0,000	0,008	0,002	0,001	0,001	0,000	0,864
3.O.Mc.	0,000	0,007	0,001	0,000	0,000	0,000	0,454
4. A.V.	0,170	0,025	0,598	0,019	0,000	0,000	0,390
5. M.T.	0,000	0,000	0,001	0,000	0,000	0,002	0,750
6.P.S.M.	0,000	0,000	0,312	0,001	0,003	0,002	0,018
7. I.L.	0,000	0,002	0,002	0,001	0,000	0,002	0,766
8. P.S.	0,000	0,006	0,002	0,001	0,001	0,001	0,210
9. S.A.	0,000	0,143	1,412	0,011	0,002	0,001	4,755
10. S.L.	0,001	0,002	0,002	0,000	0,000	0,000	1,320
11. C.O.	0,000	0,003	0,001	0,000	0,000	0,002	0,654
12. P.V.	0,000	0,007	0,002	0,000	0,000	0,000	0,430
13. T.O.	0,000	0,007	0,002	0,000	0,000	0,000	1,720
14. B.I.	0,000	0,003	0,004	0,000	0,000	0,000	0,154
15. B.I.F.	0,000	0,004	0,006	0,000	0,000	0,000	0,120
16. T.M.	0,000	0,018	0,013	0,000	0,000	0,000	0,055

Table 2. Descriptive statistics for LPS positive bacteria

Statistics

Valid	16	16	16	16	16	16	16
Missing	0	0	0	0	0	0	0
Mean	.01069	.03263	.18944	.02981	.00044	.00056	.79244
Median	.00000	.00450	.00200	.00000	.00000	.00000	.44200
Std. Deviation	.042484	.078385	.394040	.110307	.000892	.000727	1.160920
Skewness	4.000	3.054	2.407	3.985	2.145	.942	2.967
Std. Error of Skewness	.564	.564	.564	.564	.564	.564	.564
Kurtosis	15.999	9.406	5.928	15.913	4.112	-.284	9.945
Std. Error of Kurtosis	1.091	1.091	1.091	1.091	1.091	1.091	1.091
Minimum	.000	.000	.001	.000	.000	.000	.018
Maximum	.170	.296	1.412	.443	.003	.002	4.755

GRAPHICAL REPRESENTATION OF VALUES FOR LPS POSITIVE MICROBIOTA

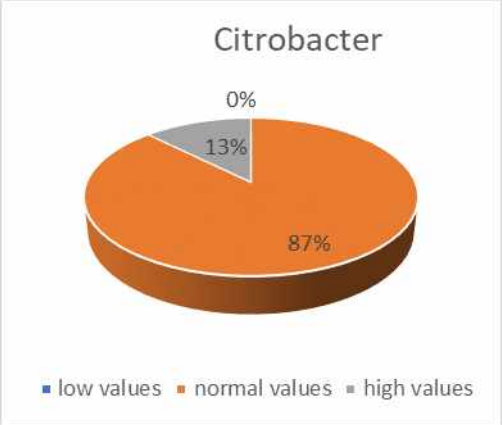

Figure 1. Graphical representation for the values of *Citrobacter spp.*

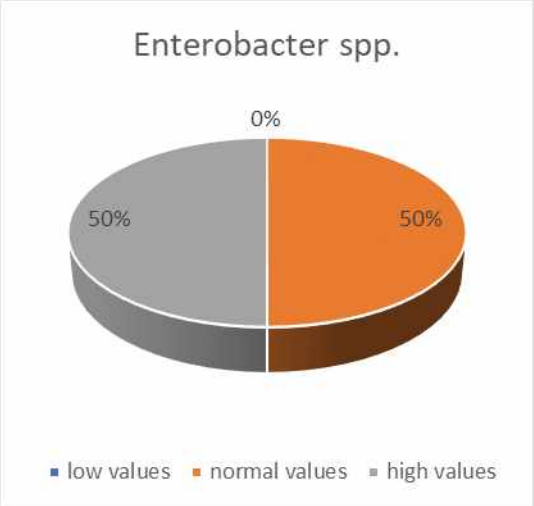

Figure 2. Graphical representation for the values of *Enterobacter spp.*

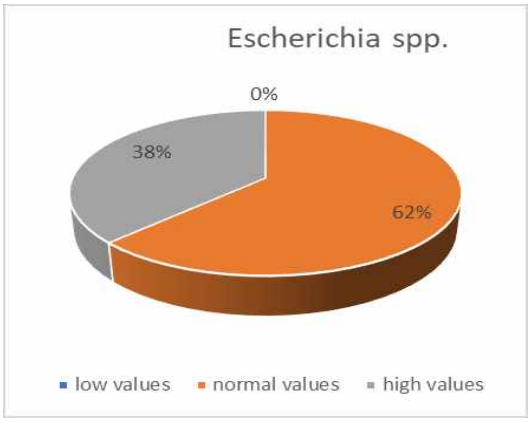

Figure 3. Graphical representation for the values of *Escherichia spp.*

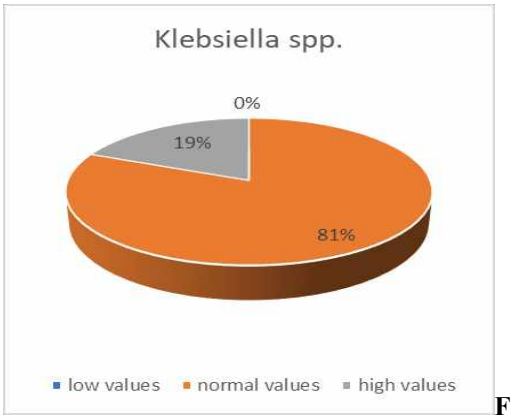

Figure 4. Graphical representation for the values of *Klebsiella spp.*

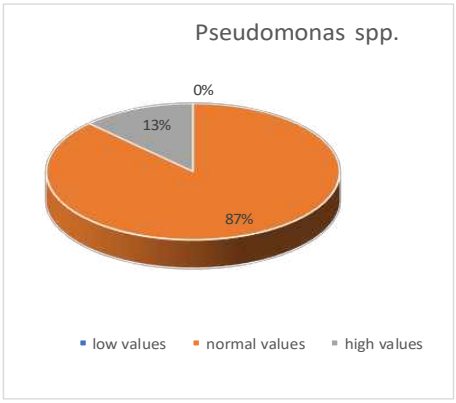

Figure 5. Graphical representation for the values of *Pseudomonas spp.*

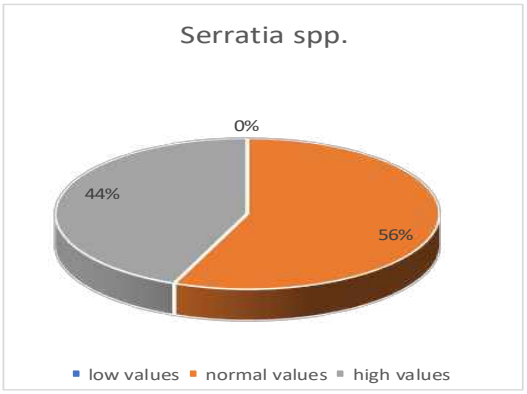

Figure 6. Graphical representation for the values of *Serratia spp.*

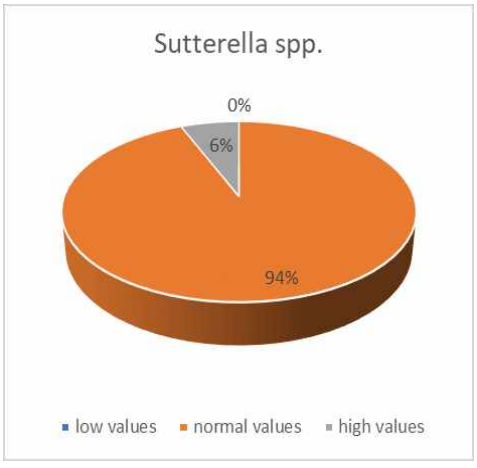

Figure 7. Graphical representation for the values of *Sutterella spp.*

Conclusions

The purpose of this research was to identify whether there is a causal link between intestinal transit disorders such as chronic constipation, the presence of behavioral disorders and the existence of an imbalance in the intestinal microbiome. Following the statistical interpretation of the results for bacteria in the intestinal microbiome, it can be observed that we obtained values outside the reference range for all bacteria analyzed. There are high values in a percentage of over 35% in the case of the bacteria: Enterobacter (50%), Escherichia (38%) and Serratia spp. (44%). Those high values may be an important factor in the worsening of autism symptoms. Children and young people with ASD participating in the research present imbalances in the intestinal microbiome. This can also be influenced by inadequate nutrition and further research should be carried out in which the emphasis is placed on the connection between nutrition and the microbiome. Due to inadequate nutrition, specific nutrients important for the efficiency of learning and social / intellectual growth may be missing. Children with autism spectrum disorder tend to have a limited food repertoire and greater reluctance to eat certain foods compared to neurotypical children. Modulating the gut microbiota in individuals with ASD with gastrointestinal disorders appears to be a promising target for future medical research.

In conclusion, it can be said that autism spectrum disorders have become increasingly common in recent years. They appear in children and persist throughout life, so early diagnosis and therapeutic intervention are recommended immediately after diagnosis. A multidisciplinary team consisting of pediatricians, psychiatrists and psychologists (specialized in autism) is needed to confirm the diagnosis and his severity. Possible treatments for autism spectrum disorders include medications that have been developed to treat other conditions that interfere secondarily with some of the symptoms of autism. Microbiome analysis can shed light on autism. Gastrointestinal disorders can affect the health of a child diagnosed with ASD, worsening certain symptoms, which leads to problems with school integration. A probiotic approach should act as a means to restore a healthy microbiota and reduce intestinal permeability. To confirm the efficacy of probiotic therapy in children with autism spectrum disorders, larger studies with laboratory analyses of the microbiota are needed for better guidance. Further research on the brain-gut-microbiome axis may lead to new methods for identifying gastrointestinal disorders in children with autism spectrum disorders and new treatments for autism spectrum disorder behavior.

R E F E R E N C E S

[1] Leaf, R., McEachin, J., Dayhorsh, J., Boehm, Marlene. (2010). ABA Therapy Guide - Behavior Modification Strategies for Autistic Children, Bucharest, Frontiera Publishing House.

[2] Mureşan, C. (2004). Infantile Autism, Psychopathological Structures and Complex Therapy, 2nd Edition. Cluj – Napoca, Presa Universitară Publishing House.

[3] Popescu, N., (1978). Dictionary of Psychology. Bucharest, Albatros Publishing House.

[4] Peeters, T. (2009). Autism: Theory and Educational Intervention, Iaşi, Polirom Publishing House.

[5] Cheng, N., Rho, J.M., Masino, S.A. (2017). Metabolic dysfunction underlying autism spectrum disorder and potential treatment approaches. Front Mol. Neurosci:34.

[6] Cucuruz, D. (2014). Autism – A Book for Parents. Bucharest, Publishing Publishing House.

[7] Boboc, D., Cojocaru, M., Roșoiu N. (2024). Autism – a reality we face. Medica Academica. ISSN 2067 - 0605. no. 147: 38-39.

[8] Adams, J., B., J., Leah, J., Powell, L., D., Quig, D., Rubin, R. A. (2011). Gastrointestinal flora and gastrointestinal status in children with autism--comparisons to typical children and correlation with autism severity. BMC Gastroenterol, 11: 22.

[9] Baquero, F., Nombela, C. (2012). The microbiome as a human organ, Clin Microbiol Infection. 18: 2–4.

[10] Proctor, L. (2019). Priorities for the next 10 years of human microbiome research. The nature: 569-623.

[11] Costache, C., Neculicioiu, N., Colosi, I. (2021). The intestinal microbiome – desirable, a lifelong alliance, Gastroenterology Journal - Microbes in the digestive system – enemies or friends, Bucharest, Medichub Media Publishing House.

[12] Boboc, D., Cojocaru, M., Gurgaş, L., Ciobică, A., Roșoiu N., (2024). Preliminary data on the link between the intestinal microbiome and autism spectrum disorders. The Medical-Surgical Journal. Iași, Romania. Vol 128 No 3 (2024): 448-460.

[13] Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota, Biochemical Journal. 474(11): 1823-1836.

[14] Barandouzi, Z. A., Starkweather, A. R., Henderson, W. A., Gyamfi, A., & Cong, X. S. (2020). Altered Composition of Gut Microbiota in Depression: A Systematic Review, Frontiers in Psychiatry, 11: 541.

[15] Boboc D., Cojocaru M, Roșoiu N., The relationship between the intestinal microbiome and the ketogenic diet in people with autistic spectrum disorders, No 134, The scientific heritage. Budapest, Hungary, 30-33, ISSN 9215 — 0365, (2024).